Chapter II

LITERATURE REVIEW

2.1 Preservation of Fresh Lime Fruit by Controlled-Atmosphere Storage

2.1.1 Introduction

A large number of complex chemical reactions, principally endothermic in characters occur in the plant. When lime is harvested and storaged, chemical and biochemical changes continue to take place. Throughout this period, lime shows a gradual reduction in quality concurrently with respiration, transpiration and a number of other biochemical and physiological changes. Eventually it deteriorates through the action of microorganism spoilage and through unfavorable enzymatic activity.

2.1.2 Respiration of Lime

During the period of growth and maturation, lime is highly dependent on photosynthesis and absorption of water and minerals by the parent plant. However, once detached, they become independent units in which respiratory process now plays a major role. The respiration process of lime can be represented by the equation.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 673 \text{ Calories.}$$

Oxygen from the air is used to burn sugar which furnishes the energy and to eliminate CO_2 as an end product.

2.1.3 Respiration Pattern

Lime is classified as a non-climacteric fruit, exhibits a non-climacteric pattern of respiration. The patterns of respiration of citrus vs. avocado; a climacteric fruit, are shown in Fig 1.(1)

The respiration pattern of lime as well as other citrus is different from the pattern shown by avocado. No climacteric rise was observed under a wide range of temperatures and rate of oxygen uptake is very low. There is no conversion of insoluble carbohydrates, such as starch, into glucose. The changes in pectic substances from the insoluble protopectin to soluble forms (pectins, pectinic acid and pectic acid) are not as clear-cut in citrus as in other fruits; which exhibit typical ripening and pass through the climacteric rise.(1)

2.1.4 Transpiration

Lime fruits continue to lose water vapor or transpiration after they are harvested. If this water loss, is not retarded, the lime fruit can become wilted, texture changes and quality of lime is reduced. Factors influencing the rate of water loss are

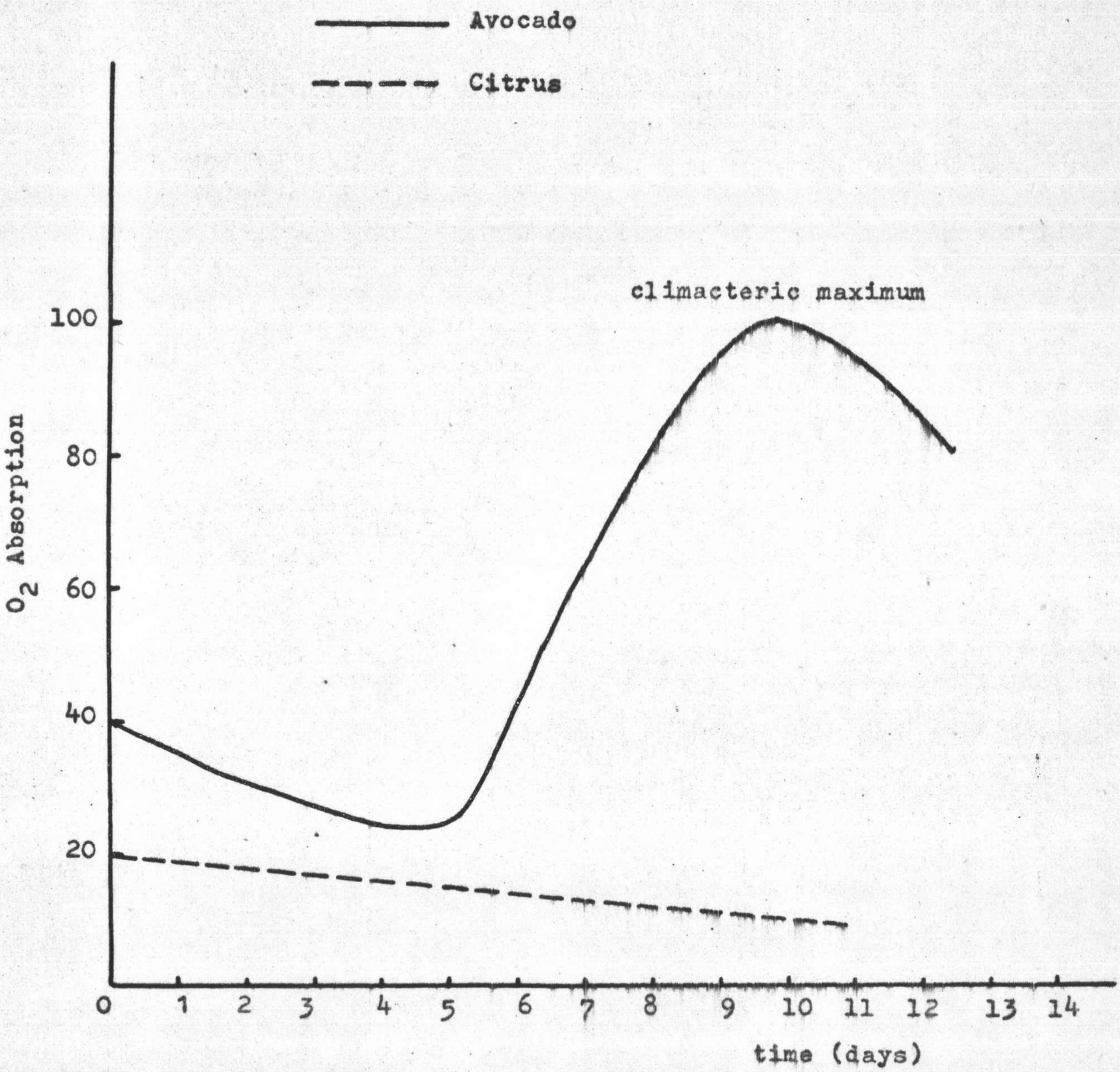

Figure 1 Patterns of respiration of citrus vs. avocado

1. Relative humidity and temperature of the air in contact with the lime.

2. Air movement. The more rapidly the air moves, the more water loss, unless the moving air is water-saturated.

3. Atmospheric pressure. The rate of evaporation of water is inversely proportional to air pressure.

The green lime freshly picked is hard, turgid and possesses a bright, shiny and attractive appearance. When the fruit is exposed to a low relative humidity it becomes dull-coloured, wrinkled and with a dry surface skin. In general, the atmosphere in storage condition should be maintained at a relative humidity not less than 85-90% and the temperature not more than 15°C (2). The fruit should also be protected from excessive desiccation by the use of water-proof cellophane or suitably waxed paper.(3)

2.1.5 Effect of Environmental Factor on Rate of Respiration

A number of factors, both internal and external are definitely known to influence the rate of respiration of lime. Under some conditions the pathway of the respiration process may be influenced by prevailing factors. Whenever the magnitude of the respiratory rate changes with a shift in one or more of the factors influencing respiration, it is evident that a change in the pathway of the respiration process has occured.

Factors that affect the rate of respiration are temperature, oxygen, carbondioxide and energy. Temperature not only affects the rate of respiration, but also affects the rates of growth of microorganisms. Therefore, the best method of preserving fresh produce after harvest is refrigeration. Generally, the rate of respiration of lime is slow at low temperature and will increase 2-3 times for every 10^oC higher. Decrease of O_2 content will affect the metabolic activity and consequently slow down the respiration rate. Chace et al (4) showed that reduction of the O_2 concentration to 10% halved the rate of respiration of lemons at 60^oF.

2.1.6 Composition Changes During Ripening

The post-harvest chemical changes that occur in the lime leading to the attainment of edible ripe stage are mitigrated through the enzymes at the expense of biochemical energy liberated through the respiration of the fruit. There is a breakdown in the pectic substances, the insoluble pectic substance to pectic acid through the activity of pectolytic enzymes. As the fruit ripening, an increase in the soluble solids of the juice rendering the fruit softening. In lime, there is a significant increase in the sugar content with a considerable decrease in the acidity of the fruit. The increase in sugar and soluble pectin is responsible for the increase in total soluble solids of the juice while together with the decrease in acidity leads to an increase in TSS/acid ratio (5).

The changes in color of the peel from green to yellow is the most obvious change. During ripening all the chlorophyll is lost, xanthophyll and carotene are synthesized (3).

There is the synthesis of volatile components which are responsible for the characteristic aroma and flavor of the lime. The changes of flavor are complex as they involve loss of acidity, loss of astringency and changes in the volatile constituents of the aroma (3).

2.1.7 Controlled-Atmosphere (C.A.) Storage of Lime

Controlled-Atmosphere storage is a system for holding produce in an atmosphere that differs substantially from normal air with respect to the proportion of nitrogen (N_2), oxygen or carbondioxide. While other gases such as carbon monoxide or ethylene, may be added. Methods of atmosphere storage were reviewed by Hall (6) who emphasized that operation of a controlled-atmosphere store required cool rooms with a high degree of gas tightener and maintain the concentration of CO_2 and O_2 at the desired levels. The benefit of C.A. storage are to prolong the storage period and shelf-life of the lime fruits and to suppress the metabolic activity or deterioration of the lime susceptible to chilling injury.

1) Effect of carbondioxide (CO_2)

The mechanism of the retarding effect of carbondioxide on ripening is still not clearly understood. Carbondioxide can extend the storage life only if injurious concentrations are avoided. The percentage of carbondioxide causing injury is directly related to the firmness of fruit and inversely related to the amount of moisture in its surface. The injury from high carbondioxide increases as the temperature is lowered for a given concentration and at a lower temperature, a given concentration may become injurious.

Kidd West (7) recommended that rate of respiration could be reduced and storage life extended by increasing the carbondioxide concentration at a suitable level at the storage temperature. Brooks et al (8) have shown that very high initial level of CO_2 can reduce fungal decay and pitting of lemons. Young, Romani and Biale (9) concluded that the high carbondioxide levels in the presence of normal oxygen concentrations stimulated respiration of the fruit. While Salama, Grierson and Oberbacher (4) concluded that increasing carbondioxide caused increased decay and deleterious effect on flavor. Grierson et al (10) working with Florida Eureka lemons concluded that, because of the high incidence of decay with increased carbondioxide levels, no worth-while benefit in keeping quality of lemons could be achieved by allowing its accumulation in storage atmosphere.

2) Effect of oxygen

Biale and Young (11) showed that the respiration rate of lemon and the loss of green colour could be significantly lowered by reducing oxygen levels and maintaining low carbondioxide concentrations. Storage of lemons in an atmosphere of 2.5% oxygen will increase respiration. Grierson et al (10) had confirmed these results and also showed that with low oxygen levels (below 2.5%) the loss of green colour was reduced;however, respiration increased after prolonged storage with concomitant fruit scalding and breakdown. This breakdown was attributed to the onset of anaerobic fermentation. Grierson et al (10) also concluded that the storage environments of about 6% oxygen helped extend the life of Florida and Californian lemons.

3 Effect of ethylene

In C.A. storage, the accumulation of ethylene is occured and ventilation is necessary. Biale (12) found that citrus infected with green mould can produce large quantities of ethylene. Craff (13) also confirmed that even high level of CO_2 did not suppress the ethylene induced respiratory response in store lemons. Ethylene is suspected with stimulating green mould growth and further ethylene production as a result of an automatic catalytic effect of accumulated ethylene in an enclosed environment (14). Under C.A. condition Biale (12) also reported that ethylene stimulated respiration of lemons, even when the fruits were held in an atmosphere of of 5% oxygen and 5% carbondioxide. The respiration rate of green lemon fruit is increased when exposed to ethylene at concentration as low as 1 /µl/l and the keeping quality of lemons was improved if storage rooms were well ventilated(15).

. Burg and Burg (16) realized the importance of ethylene in the C.A. environment and recommended that the ideal atmosphere for preserving fruit after harvesting was one which should contain 5 to 10% CO_2 and 1 to 30% O_2 and as little ethylene as possible. Fidler (19) concluded that ethylene appears to be less effective at low temperature.

4. Other important factors related to controlled-atmosphere storage

Controlled-Atmosphere storage is applied to the storage of fruit in an atmosphere of oxygen and carbondioxide. Successful results led to atmosphere control being combined with temperature control, humidity control and control of decay by microorganisms.

a) Temperature

Temperature affects the rate of respiration and reduce the rate of growth of microorganism. The reduction of storage temperature to a minimum without inducing chilling injury or other physiological disorders has an effect on improving the storage life.

Optimum storage temperature for lime vary between 9°C and 12^oC depending on the duration of storage (18). Extended storage at lower temperature induces physiological breakdown and decay in the lime. Chilling injury will produce rind breakdown, rind pitting, and scald sunken portions of the rind are sharply delimited and range in the peel colour from tan to dark brown. The disorder does not extend into the flesh but does render fruit more readily subject to decay by fungi. The rind breakdown is likely to develop at temperature below 10^oC (50^oF). Such temperatures are frequently employed to keep limes green and to check decay (19). Braverman et al (20) reported that after storage of lime at 4.5^oC, chilling injury appeared in the form of peel injury which showed as dark sunken surface at 5 weeks storage.

b) Decay of lime by microorganisms

Main disease and injury of lime occurred during the storage were green and blue mold and stem end rots (20). Blue and Green mold disease of citrus are caused by <u>Penicillium italicum Wehmer</u> and <u>Penicillium digitatum Sacc</u>. The special characteristics of blue mold are a narrow, while margin of fungus growth beyond the blue sporulation zone and a zone of water soaked rind tissue surrounding the mold, decay by blue mold is softer and more easily broken by pressure. Moreover, blue mold spreads from fruit to fruit by contact more readily than does green mold.

Green mold has a wider white margin with a more pasty appearance than that of blue mold, and the invaded fruit tissue beyond the white band of mycelium appears less water-soaked and is firmer.

Usually both fungi can be recovered from decaying fruit even though green mold may completely over grow the other and be the only one evident. The indication of the presence of both fungi without actually culturing and isolating then, is red discoloration in the rind albedo (19).

Stem and rot caused by *Diplodia natalenois* and *phomopsis citri*. These molds infect lime buttons and penetrate the central albedo areas and then spread through the remainder of the lime.

c) Control of mold and rot with fungicides

Many chemicals have been test to evaluate their fungicidal activity in pre-and post-harvest treatments. These important chemicals are shown to be effective fungicides:

i) Thiabendazone (TBZ): 2,4-Thiazolylbenzimidazole was tested for its fungicidal activity against *Penicillium* sp. and considered to be the most effective (21).

ii) Benlate: Methyl 1-(butycarbamoyl)-2-bengimidazole-carbamate was moved to be the most effective systemic fungicide now available for the commercial control of spoilage from *Penicillium italicum* and P. *digitatum* of citrus fruits. However, a surfactant has to be used along with Benlate because it congeals when the water or the fungicide is cold, and requires intense agitation for dispersion (22).

iii) Thiophanate methyl was teste by Vanderweyen et al (23) for its fungicidal activity in comparison with TBZ and Benlate and was found to be equally effective as the other two chemicals in the control of green molds.

2.1.8 Research Work on Controlled-Atmosphere Storage of Lime

Experiments of C.A. storage of citrus fruits have been conducted mostly on lemons, but very little work has been done on lime for many years. Salama, Grierson, and Okerbacher (24) found that many modification of the storage atmosphere increased decay in Florida limes. Tipayang (25) reported that limes stored for 3 months in the atmosphere of 10% O_2 and 5% CO_2 at 15^oC were still in very good condition. Rugg, Gil. and Wills, A.W.(26) reported that storage of lemons in the atmosphere of 10% O_2, 5% CO_2 at temperature of 15^oC could keep the lemons for 22-23 weeks. Young et al, (9) subjected lemons to controlled-atmosphere storage wherein 0.5, 5% and 10% CO_2 and atmosphere of 5, 10 and 21% O_2 were used. It was observed tnat CO_2 led to an unprecedented stimulation in respiration which was more striking at 10% CO_2 level than at 5% and more in combination of CO_2 with air than with O_2 concentration below air. However, the storage life of lemons was prolonged by CO_2 especially at lower levels of O_2.

Kajiura and Iwata (27) concluded that no optimum O_2

concentration could be suggested, owing to the development of low temperature injury at 4°C. In their further study (28). Kajiura and Iwata observed that controlled atmospheric storage at oxygen concentration below 3% led to a development of fermented flavor in the fruits.

Spalding et al (29) found that non to slight benefit from C.A. storage of 'Persian' lime because they developed severe injury and decay in low O_2 atmosphere.

2.2 Lime Juice Concentrate Processing

2.2.1 Introduction

Concentration of citrus juices have been done for many years, but the low temperature evaporation methods i.e. vacuum concentration and freeze concentration have been developed recently. (34) Heikal et al (35) concluded that concentrating lime juice under vacuum up to 42% total soluble solid was the most suitable concentration method. Hall (36) studied the effect of concentration on lemon juice flavor. He reported that the juice prepared from vacuum evaporator and freeze concentrated sample did not have significant level of oxidative off-flavor or aroma. Pruthi et al (37) did the research on cashew apple concentrated juice and found that the important physico-chemical characteristics like ascorbic acid, pH, color and flavor of vacuum concentrated cashew apple juice were better than open-pan concentrated cashew apple juice.

2.2.2 Quality of Concentrated Lime Juice during Storage

The type of changes in important physical and chemical properties of lime juice concentrate during storage are

2.2.2.1 Browning, The concentrated lime juice can develop browning color during storage. It has been reported to be a non-enzymic browning reaction in which ascorbic acid, citric acid, amino acid and oxygen may contribute to the reaction.

As for the effect of ascorbic acid on the development of browning, Koppanyi et al (38) reported that dehydroascorbic acid but not ascorbic acid, reacted in solution at 98.8^{o}C with amino acids to form strongly colored compounds. Clegg (39) found that browning in lemon juice was related to amino acid-sugar interactions, and added that the principal rate of amino acids was to increase the browning potential after the oxidation of its ascorbic acid. Clegg also shown that the degree of non-enzymic browning was influenced by the pH value of the product and was maximal at pH 4.5. Ascorbic acid in lemon juice exhibited maximal browning at pH 4.5 in the presence of citric acid.

Heikal et al (40) concluded that reducing sugars, especially glucose seemed to play a certain role in the browning of lime and orange juices. Storage conditions especially temperature, have a definite bearing on the role of the reaction in the browning of lime and orange juices.

Curl (41) found that browning increased during storage at room temperature. Meanwhile, Pederson et al (42) studied in some detail the darkening or browning in some juices during storage at various temperature. They found that a soluble brown compound was formed in all samples stored at various temperatures. They also found a marked decrease in color of citrus juices, using the optical density as an index in evaluating the color of such juices.

Oxygen can react with ascorbic acid in the presence of trace amount of copper to give dehydroascorbic acid and hydrogen peroxide. The hydrogen peroxide formed during this reaction can lead to further oxidation of ascorbic acid either directly or indirectly, by breaking down to water and oxygen, both reactions being catalysed by cupric ion (43). Dehydroascorbic acid retains full ascorbic acid activity but is more thermolabile than ascorbic acid and would therefore be easily destroyed during heat processing.

2.2.2.2 Destruction of ascorbic acid. The destruction of ascorbic acid during storage was reported by many research workers (39,43,44). Joslyn and Tressler (34) reported that single-strength juices, and especially in concentrates, much more ascorbic acid disappeared on room temperature storage that could not be accounted for by the oxygen originally present. This disappearance of ascorbic acid was considered to be among the important factors in forming the colored compound in the juices. Moored et al (45) found that the retention of ascorbic acid of citrus juices stored in tin had a higher retention than the corresponding pack in glass. Oxygen in head space of the can had a detrimental effect on the ascorbic acid concentration in de-aerated sweetened orange juice.

2.2.2.3 Off flavor. Off-flavor can be developed from the oxidation of the lipid fraction or a change in the essential oil. Limonene, an important essential oil, will decrease during high temperature storage.(46) The off-flavor which develops during storage may make the lime juice unacceptable to the consumer. Underwood and Rockland (47) in their work on citrus fruits indicated that amino acids-sugars interactions may be important in the darkening and development of off-flavor in citrus products.

2.2.3 Use of Sulphur Dioxide in Lime Juice

Sulphur dioxide is more effective against mold spores, yeast and bacteria. It inhibits browning of the juice and help in reducing losses of ascorbic acid. It is used to prevent the oxidation of essential oils, carotenoids and consequent development of off-flavor and loss of color in citrus juices. It has no effect on total acidity analysed as citric acid (48) as well as no effect on pectic enzymes which are responsible for the breakdown of tissue or which cause loss of cloud in citrus juices.

The amount of sulphur dioxide used depends on the pH of the juice and the amount of suspended pulp. Clarified lime juice can be preserved safely with little as 350 ppm, but pulpy orange juice requires as much as 1500 ppm.(49) The use of sulfites is limited by the fact that at residual levels above 500 ppm, the taste begins to be noticeable. Sulphur dioxide is the only chemical preservative which can be removed from the juice by applying heat or vacuum.

According to the results from the Department of Science, Ministry of Industry,(50), it was found that single-strength lime juice with 1000 ppm of potassium metabisulfite as preservative could be kept at 24°C for one year without much change in qualities except for the decrease in ascorbic acid content to zero at the end of second month, but it was still acceptable. When 500 ppm of sodium sorbate or sodium benzoate were used, the juice could be kept only for half a month and its color turned brown. Heikal et al (51) reported that concentrations varying from 100-200 ppm sulphur dioxide are the most suitable for lemon juice preservation.